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Calcitriol does not significantly enhance the efficacy of radiation of human cervical tumors in mice

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Summary

Objective: Calcitriol can enhance the sensitivity of cancer cells to radiation *in vitro*. The authors aimed to investigate the potential synergistic effect of calcitriol and radiation in a xenograft mouse model of human cervical cancer. *Materials and Methods:* Tumor-bearing mice were fed with vehicle arachis oil or 2.5 μg/kg calcitriol daily for 15 consecutive days. Some mice received ten Gy radiation on day 7 post treatment. Tumor growth was monitored, and the tumor tissues were examined by histology and electron microscopy. *Results:* Treatment with either calcitriol or radiation significantly inhibited the growth of implanted cervical cancers ($p < 0.05$ *vs.* control) and increased the number of dead tumor cells in the tumor sections. However, there was no significant difference in the tumor weights between the mice with radiation alone and both radiation and calcitriol treatment. *Conclusion:* Calcitriol had anti-tumoral activity, but failed to enhance the efficacy of radiation in human cervical cancers.

Key words: Calcitriol; Cervical tumor; Radiation response.

Introduction

Cervical cancer is one of the more common malignancies and a leading cause of cancer-related morbidity and mortality in women worldwide. Cervical cancer is estimated to affect 529,800 women annually [1]. Currently, patients with localized advanced cervical cancer are usually treated with external beam radiotherapy (EBRT), concomitant chemotherapy, and brachytherapy (BT) [2]. These therapeutic strategies have improved significantly in the local control of tumor progression and the survival of patients with cervical cancer [3]. The prognosis of patients with advanced recurrent cervical cancer depends mainly on treatment and on the site and extent of recurrence [4, 5]. However, the currently used medicines for chemotherapy, such as platinum-based chemoradiation regimens, usually cause severe side-effects and are not well tolerated in some patients [3]. Therefore, the discovery of new safer medicines for the treatment of patients with advanced cervical cancer will be of great significance.

A previous study has revealed an inverse association between vitamin D intake and cervical neoplasia risk in Japanese women [6]. Calcitriol, 1,25-dihydroxyvitamin D3 (1,25- $(OH)_2D_3$,), the biologically active metabolite of vitamin D, has been shown to regulate the growth of various types of cancer cells [7, 8]. Moreover, accumulating evidence indicates that calcitriol inhibits the growth of several cancer cells, including breast cancer cells [9]. Calcitriol can induce cell cycle arrest and apoptosis, and inhibit tumor cell invasion, metastasis, and angiogenesis [10]. Treatment with calcitriol increases the sensitivity to ionizing radiation in breast and prostate cancer cells [11, 12]. However, little is known about the impact of treatment with calcitriol on the sensitivity to radiation in human cervical tumors.

In this present study, the authors investigated the effects of calcitriol or combined radiation therapy on the growth of implanted human cervical tumors in mice. Surprisingly, they did not find that treatment with calcitriol enhanced the sensitivity of human cervical tumors to radiotherapy *in vivo*. They discussed the potential reasons for the failure.

Materials and Methods

Cell culture

Human cervical carcinoma HeLa cells were provided by the Experimental Center of the Second Affiliated Hospital of Harbin Medical University, Heilongjiang, China. The cells were maintained in high glucose Dulbecco's Modified Eagle's medium (DMEM) supplemented by 10% fetal bovine serum at 37°C in a 5% CO_2-humidified incubator.

Establishment of tumor-bearing nude mice model

Female BALC/c nude mice at four to five weeks of age and weighing 20-22 grams were obtained from a Shanghai Animal Laboratory in China. The animals were housed under a specific pathogen-free facility and had free access to food and water. Every effort was made to minimize the numbers and suffering of animals used in the experiments. The experimental protocols were approved by the Animal Ethical Committee of the Second Affiliated Hospital of Harbin Medical University, and the work was undertaken within which and conformed to the provisions of the Declaration of Helsinki.

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HeLa cells at logarithmic growth phase were harvested, and the cells (10^7 cells in 0.1 ml of PBS per mouse) were implanted subcutaneously into the lateral thigh of the posterior limb of individual mice. After tumor cell inoculation, the development of solid tumors in mice was monitored. Two weeks after incubation, the tumors reached an appropriate five mm in one dimension, and the animals were used for the following experiments.

Experimental assignment and treatments

The tumor-bearing nude mice were randomized and fed with 0.2 ml of arachis oil or with 0.2 ml of arachis oil containing 2.5 μg/kg of calcitriol by gavage daily for 15 consecutive days. Some mice from each group received ten Gy radiation using a 6MV X-ray at a focus-surface distance of 100 cm and a dose rate of two Gy/min once at day 7 post calcitriol treatment. The growth of implanted tumors in the vehicle alone control, radiation, calcitriol alone, and combined calcitriol and radiation groups (n=5 per group) of mice were monitored every five days up to 15 days post-initial treatment using a vernier caliper in a blinded manner. The tumor volume (V) was calculated by the following formula: $V=1/2\times a\times b^2$ (a was the length and b was the width of tumor). At the end of the experiment, the mice were sacrificed and their tumors were dissected and weighed. The tumor growth inhibition rate was calculated using the following formula: Tumor growth inhibition (%) = (Tumor weight in control group – Tumor weight in treatment group) / Tumor weight in control group × 100%.

Histological examination

The dissected tumor tissues were fixed in 4% paraformaldehyde (PFA), dehydrated with a graded ethanol series, and embedded in paraffin. The tumor tissue sections (five μm) were deparaffinized and rehydrated. Subsequently, the sections were stained with haematoxylin and eosin (HE) and examined under a light microscope. Images were captured by Image-pro plus software.

Transmission electron microscope (TEM) analysis

For TEM analysis, tumor tissues were fixed with 2.5% glutaraldehyde and post-fixed with 1% OsO_4. After dehydration through a graded ethanol series and acetone, the tumor tissues were embedded in Epon812 for ultra thin sectioning. The ultra thin sections were then stained with uranyl acetate and lead citrate, and examined under a transmission electron microscope.

Statistical analysis

Data shown are representative photoimages or expressed as the mean ± standard deviation (SD). The difference among the groups of mice was analyzed by analysis of variance (ANOVA) using SPSS 10.0 software. A p value < 0.05 was considered statistically significant.

Results

To determine the effect of treatment with calcitriol on the sensitivity of human cervical cancer to radiation, BALB/c mice were inoculated with human cervical cancer HeLa cells to induce solid tumors. When the mice developed tumors, they were randomized and fed with vehicle or calcitriol and some of mice were subjected to radiation. All of the animals survived 15 days after initial treatment. The tumor-bearing control mice, but not the mice, which received radiation or calcitriol, showed decreased appetite, decreased activity, and weight loss at five days post-treatment. As shown the Figure 1, calcitriol, radiation alone, or combined therapy significantly mitigated the loss of body weight in animals on day 10 and day 15 post-treatment. Treatment with either calcitriol or radiation alone significantly inhibited the growth of implanted human cervical tumors (Figure 2A). However, treatment with both calcitriol and radiation did not significantly enhance the inhibition of tumor growth in mice ($p > 0.05$ *vs.* the radiation alone). Similarly, treatment with either calcitriol or radiation alone significantly reduced the weight of the dissected tumors, but both treatments had no obvious synergistic effect on reducing the size of tumors in the mice (Figure 2B).

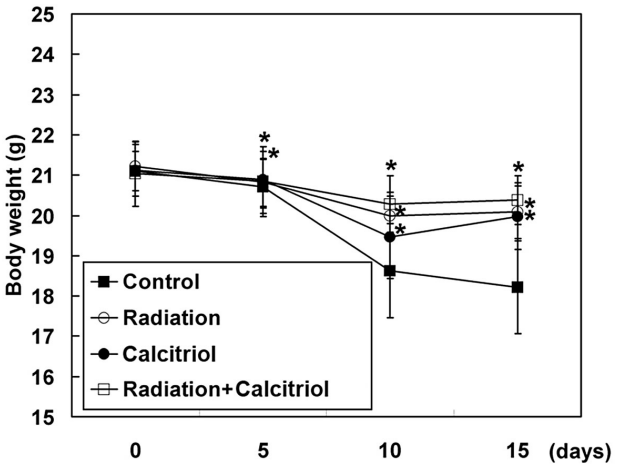

Figure 1. — Treatment with either calcitriol or radiation mitigates the loss of body weight in tumor-bearing mice. BALB/c nude mice were inoculated with Hela cells to establish human cervical cancer, and the mice were randomly treated with calcitriol or vehicle. Subsequently, some mice from each group were subjected to radiation and their body weights were measured at the indicated time points post treatment. Data are expressed as the mean ± SD of the body weights of each group (n=5) of mice *$p < 0.05$ *vs.* the control mice treated with vehicle alone.

The authors next characterized the morphology of tumor tissues from different groups of mice by histological examination. While typical tumor structure and morphology, proliferative fibrosis, and inflammatory infiltrates were observed in the tumors from the controls mice, there were obviously less infiltrates and fibroblast proliferation as well as many dead tumor cells in the tumors from both the mice receiving radiation and calcitriol (Figure 3). There was no obvious difference in the tumor morphology from the mice that had been treated with radiation alone or combined with calcitriol. Further TEM analysis revealed that nuclear condensation, chromatin margination, and membrane damage were observed in the tumors from the mice that had been treated with radiation or combined with calcitriol, but not from the control or calcitriol alone-treated mice (Figure 4).

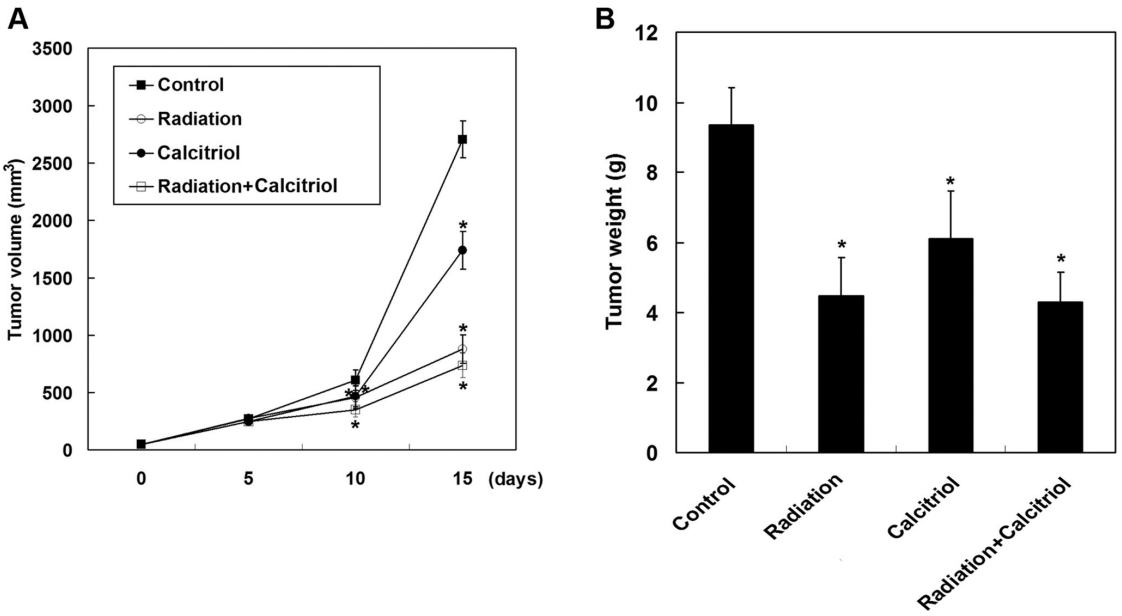

Figure 2. — Treatment with either calcitriol or radiation inhibits the growth of implanted cervical cancer in mice. Following treatment with calcitriol, the growth of implanted tumors was monitored at the indicated time points up to 15 days post-treatment. At the end of the experiment, the tumors in individual mice were dissected and weighed. Data are expressed as the mean ± SD of the tumor sizes or weights in different groups of mice (n=5 per group). (A) The growth of implanted tumors; (B) the tumor weights. *$p < 0.05$ *vs.* the control mice treated with vehicle alone.

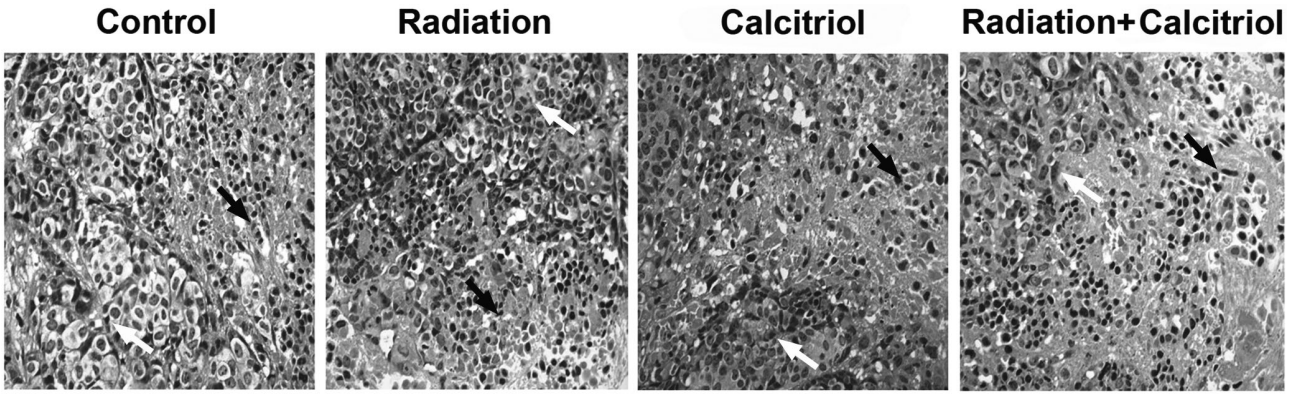

Figure 3. — Histological analysis of tumors. At the end of the experiment, the tumor tissues from individual mice were dissected out and subjected to histological examination by HE staining. Data shown are representative photoimages of the implanted cervical tumors from individual groups of mice (n=5 per group). Scale bar: 100 μM; The black arrows: inflammatory infiltrates; The white arrows: dead tumor cells.

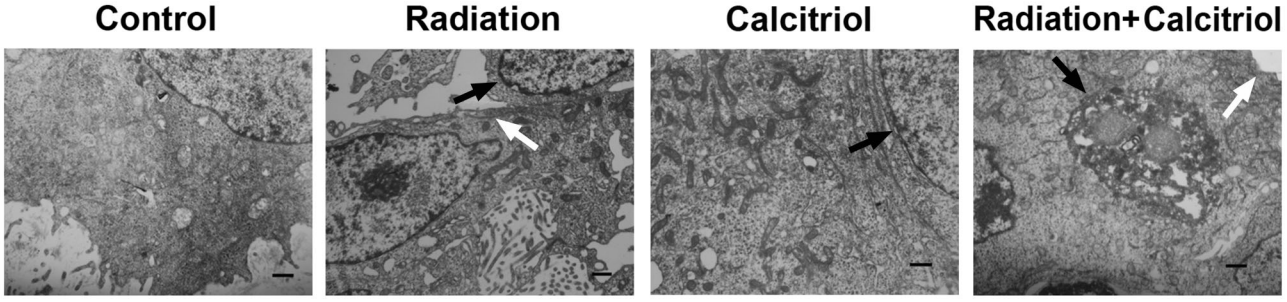

Figure 4. — TEM analysis of tumors. The tumor tissues from individual mice were dissected and subjected to TEM analysis. Data shown are representative photoimages of the implanted cervical tumors from individual groups of mice (n=5 per group). Scale bar: 500 nm; The black arrows: nuclear condensation; the white arrows: membrane damage.

Together, these data suggested that treatment with calcitriol inhibited the growth of human cervical tumors in mice, but failed to enhance the sensitivity of human cervical cancer to radiation.

Discussion

In this present study, the authors examined the potential role of calcitriol in radiation responses in cervical tumor-bearing nude mice. Their results indicated that, although radiation, calcitriol alone, or combined therapy significantly inhibited the tumor growth *in vivo*, there was no synergistic effect of combined therapy on inhibiting the growth of human cervical cancer in mice.

Previous studies have shown that calcitriol has anti-proliferative, anti-inflammatory, pro-differentiation, and pro-apoptotic activities in many human cancers [7, 10, 13, 14]. Evidentially, treatment with calcitriol inhibits the growth and promotes apoptosis of breast cancer cells *via* induction of reactive oxygen species (ROS) [9]. Treatment with calcitriol also induces the apoptosis of ovarian cancer cells through the down-regulation of telomerase [15] by modulating MiR-498 expression. Consistent with these observations, the present findings indicated that treatment with calcitriol greatly suppressed the growth of human cervical tumor in nude mice. It is possible that calcitriol may also induce human cervical cancer cell apoptosis *in vivo*. Indeed, the authors obviously observed increased dead cells and apoptotic features in the tumor section from the calcitriol–treated mice. Although the presence of vitamin D receptor (VDR) in cervical tumors remains controversial, increased levels of VDR expression were detected in cervix carcinomas, as compared with that in normal corresponding tissues [16]. Furthermore, increased levels of VDR were also detected in the cervical cancer tissues from human patients, although there was no statistically significant correlation between the levels of VDR expression in the cancers, anti-Ki-67 or anti-p53 staining, and histopathological data (tumor stage, lymph node status, grading, histological tumor type) [17]. On the contrary, there VDR expression was detected in another study of one specimen [18]. The present authors are interested in further investigating the mechanisms underlying the action of calcitriol in inhibiting the growth of human cervical cancer, including measuring the VDR expression.

Radiotherapy represents an effective treatment modality for patients with cervical cancer [2]. However, many patients with advanced cervical cancer respond poorly with tumor progression and recurrence [4, 19]. Adjuvant chemotherapeutic agent may enhance radio-sensitization of cervical tumor cells by direct toxicity against tumor cells or by inhibiting radiotherapy-related repair in the tumor [20, 21]. Although cisplatin-based chemotherapy increases the sensitivity of cervical cancer to radiotherapy, this therapeutic strategy does not prolong the survival of patients with cervical cancer due to severe side-effects [22]. Previous studies have shown that treatment with calcitriol enhances the sensitivity of different types of human cancers to radiotherapy [7, 10, 13, 14]. However, the present authors found that treatment with calcitriol failed to enhance the sensitivity of human cervical cancer to radiation in mice. These data suggest that different types of human cancer may have various responses to calcitriol treatment. They are interested in further investigating the mechanisms underlying the failure of calcitriol treatment in radiotherapy for human cervical cancer.

Conclusion

In summary, the present study indicated that treatment with calcitriol and radiation alone significantly inhibited the growth of human cervical cancer in mice. However, treatment with calcitriol failed to enhance the sensitivity of human cervical cancer to radiotherapy in mice.

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